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The objective of the project is to establish and use an *in vitro* system of intact hepatocytes from rodent and human liver to develop quantitative data on the metabolism of toxic chemicals that can be used in risk assessments. Currently, two different *in vitro* models are most frequently used for comparative metabolism studies—isolated hepatocytes and precision-cut liver slices. In the second year of the project, hepatocytes and liver slices from rat and human liver were compared to determine their capability to metabolize chloroform and a model substrate, 7-ethoxycoumarin. The 7-ethoxycoumarin O-deethylation activity of hepatocytes was consistently higher than the activity measured with liver slices, when standardized by protein content of the two preparations. The metabolism of chloroform by hepatocytes isolated from male F344 rats was characterized by analyzing headspace and medium concentrations. The kinetic constants, estimated from the headspace data generated in these experiments, are $K_m = 34$ nmol/flask and $V_{max} = 24$ pmol/min/ 10^6 cells. Headspace analysis was also useful for determining chloroform metabolism by liver slices. After addition of 3.6 nmol/flask, the rate of chloroform loss was 2.5 pmol/min/mg tissue.

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IN VITRO SYSTEM FOR STUDYING METABOLISM OF ENVIRONMENTAL CHEMICALS IN HUMAN CELLS

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Carol E. Green	Principal investigator
Jack E. Dabbs	Project leader: hepatocyte isolations, liver slice preparation, incubations, toxicity measurements
G. Ross Gordon	Gas chromatographic analysis
Valorie Weizer	Gas chromatographic analysis

OBJECTIVES

The objectives of the project are to establish and use an *in vitro* system of intact isolated cells from rodent and human tissues to develop quantitative data on the metabolism of toxic chemicals that can be used for risk assessments. The following halogenated aliphatic solvents are being studied: chloroform, 1,1,1-trichloroethane, trichloroethylene, dichloromethane, bromochloromethane, and carbon tetrachloride. These compounds are used first with rat liver preparations to establish the conditions for generating kinetic constants for metabolism based on disappearance of the parent compound. Isolated hepatocytes and precision-cut liver slices are being compared to optimize the correspondence between the *in vitro* results obtained and published *in vivo* data. The system developed with rat liver will then be applied to human liver preparations. The same set of halogenated solvents will be studied, and the resulting data will allow quantitative comparison of the metabolism and cytotoxicity in these species. These data will be analyzed to characterize interspecies differences in the kinetics of metabolism.

MATERIALS AND METHODS

HEPATOCYTE ISOLATION

Hepatocytes were isolated from male F344 rats (300-450 g) by the whole-liver perfusion method (Green et al., 1983). The cell yield and viability were determined by counting the cell suspension using a hemacytometer and calculating the percentage of cells that excluded trypan blue. Hepatocytes were also isolated from human liver specimens using the biopsy perfusion technique (Allen and Green, 1993).

Isolated hepatocytes were suspended in a modified Waymouth's 752/1 culture medium (CMH) that contained 11.2 $\mu\text{g/ml}$ alanine, 12.8 $\mu\text{g/ml}$ serine, 24.0 $\mu\text{g/ml}$ asparagine, 84.0 $\mu\text{g/ml}$ gentamicin sulfate, 0.168 $\mu\text{g/ml}$ aminolevulinic acid, 5.0 $\mu\text{g/ml}$ oleic acid, 5.0 $\mu\text{g/ml}$ linoleic acid, 1.0 $\mu\text{g/ml}$ d,l-tocopherol, 288 ng/ml testosterone, 272 ng/ml estradiol, 393 ng/ml dexamethasone, 7.9 $\mu\text{g/ml}$ thyroxine, 30 ng/ml glucagon, 0.02 U/ml insulin, and 0.2% BSA.

PREPARATION OF PRECISION-CUT LIVER SLICES

Liver cores were prepared from either rat liver or a dissected lobe of the human liver using a sharpened cylindrical tube attached to a drill press. A core (about 2 cm in length) was then placed in the tissue holder of the Krumdieck slicer (Krumdieck et al., 1990). Slices were then prepared with a diameter of about 1 cm and a thickness of approximately 250-300 μm . Slices were incubated in dynamic organ culture (Smith et al., 1986). Two or four slices were loaded onto each mesh insert and placed in scintillation vials for a preincubation period at 37°C for 1 hr, using the same culture medium as for the hepatocytes. The medium was then aspirated and replaced with fresh medium (2.0 ml/vial).

INCUBATION OF HEPATOCYTES AND LIVER SLICES WITH HALOGENATED SOLVENT

The hepatocytes were prepared at a density of $4 \times 10^6/\text{ml}$, and 4 ml of cell suspension was added to each side-arm incubation flask. The flasks were matched for total volume (about 25 ml) and outfitted with gas-tight valves to allow repeat sampling of either the head space or the medium (Mininert valves, Pierce, Rockford, IL) to fit both the top and side-arm opening of the flask. After addition of the cells, the flasks were gassed vigorously for about 30 s with 95% air:5% CO_2 and immediately stoppered. The solvent (chloroform in the studies described in this report) was added with a Hamilton

syringe through the Mininert valve directly into the medium. The flasks were placed in an oscillating water bath and maintained at 37°C at 60-70 osc/min.

The liver slices (4 slices per vial or between 130-200 mg of tissue) were placed on a screen mesh support and inserted into a scintillation vial. The vials contained 2 ml of the same medium used with isolated hepatocytes and were gassed with 95% air:5% CO₂. They were placed in a rotating rack at 37°C, which allowed the slices on the support mesh to roll freely through the medium. After a preincubation period of 1 hr, the medium was changed, the flasks were gassed again, and the test chemical (chloroform) was added through the gas-tight valve.

The stock solutions of chloroform were prepared by dissolving the halogenated hydrocarbon in DMSO and then diluting it into CMH in a sealed vial. Stock solutions were prepared to give the following concentrations of chloroform: 7200, 720, 72, and 7.2 nmol/ml. Aliquots of these solutions were added directly to the media of the incubation vessels (0.1 ml to hepatocytes and 0.05 ml to liver slices).

Media samples for analysis were removed through the side-arm valve with a Hamilton syringe, and air samples were removed through the top valve with an air-tight syringe. Air samples were injected directly into the gas chromatograph. Media samples (0.1 ml or 0.05 ml) were added to vials containing saturated urea (0.6 ml) and stored on dry ice until analysis. The vials were heated to 60°C and then chloroform was analyzed in the headspace (Pryor et al., 1991).

GAS CHROMATOGRAPHIC ANALYSIS OF CHLOROFORM

Chloroform was analyzed using gas chromatography with a 2 mm × 6 ft glass column packed with 0.1% SP1000 on 80/100 Carbopack C using a Varian Model 3700 gas chromatograph equipped with an electron capture detector and a Hewlett-Packard Model 3390A integrator. The following parameters were used: column temperature, 125°C; detector temperature, 150°C; injector, 250°C; and a N₂ flow rate of 30 ml/min.

Media aliquots (0.05 ml) were removed from the closed flasks through a Mininert push-button valve and added to a 1-dram vial (15 × 45 mm, 4.9 ml) through a Teflon-lined septum. The vial contained 0.6 ml of saturated urea and was maintained on dry ice before and after the addition of the media aliquot. Chloroform was released into the vial headspace after allowing the vial to thaw at room temperature and then heating it to 60°C for 10 min. The headspace of the vial was then sampled with a gas-tight syringe and injected in the gas chromatograph.

7-ETHOXYCOUMARIN O-DEETHYLATION ACTIVITY

As a quick method for comparing the relative metabolic activity of hepatocytes and liver slices, 7-ethoxycoumarin O-deethylation (ECOD) activity (a cytochrome P450 associated activity) was determined. The preparations were incubated with 100 μ M 7-EC for 1 hr. The culture medium was assayed for hydroxycoumarin production using the fluorometric method of Greenlee and Poland (1978). One aliquot of medium was assayed directly, and a second aliquot was assayed after the conjugates were cleaved with β -glucuronidase and sulfatase. ECOD activity was calculated as the amount of hydroxycoumarin produced/hr/mg protein. The fraction of the total hydroxycoumarin that was conjugated by the preparations was also determined.

PROGRESS AND RESULTS

The purpose of the current experiments is to extend previous work conducted at SRI on the cytotoxicity and metabolism of chlorinated solvents (Tyson et al., 1983; Knadle et al., 1990). In those studies, the dose-response relationship for cytotoxicity of several chlorinated solvents to rat hepatocytes was established (Tyson et al., 1983) using relatively high concentrations and short exposure periods. For example, the EC_{50} (concentration at which 50% of the cellular LDH was released to the medium in 2 hr) for chloroform was calculated to be 7.1 ± 0.49 mM. At these concentrations, a decrease in chlorinated hydrocarbon level in the medium was not be detected. The metabolite profile of another chlorinated hydrocarbon, trichloroethylene, was well-characterized in our laboratory using rat and human hepatocytes (Knadle et al., 1990) to compare the disposition of this solvent in the different species and to establish a basis for using the formation of particular metabolites as a means for predicting hepatocarcinogenic risk to humans. In that study, hepatocytes from both species metabolized trichloroethylene to trichloroethanol and its glucuronide, chloral hydrate, and trichloroacetic acid. The rate and extent of metabolism was greater in rat than in human hepatocytes. The pattern of metabolites also varied with species in our study. Rat hepatocytes formed proportionally more trichloroacetic acid, the metabolite believed to be responsible for the hepatocarcinogenicity of trichloroethylene (Elcombe, 1985). Although kinetic constants were not calculated from the experiments with trichloroethylene, it was apparent that metabolism by rat hepatocytes became saturated at a higher concentration than did the human hepatocytes.

Our goal in the current research project is to establish an *in vitro* technique for readily determining kinetic constants for metabolism of chlorinated solvents using tissues from both laboratory species and humans. Since the literature suggests that the K_m values for the chlorinated solvents that we plan to study are in the micromolar range (Gargas et al., 1986), it was necessary to establish new techniques that allow the investigation of relatively low concentrations.

Although we initially planned to use isolated hepatocytes in suspension for these studies, new data led us to consider whether the basic *in vitro* model for these studies should be modified. We have observed in other experiments that the viability of human hepatocytes in suspension culture decreases much faster than that of rat liver cells. On the average, in rat hepatocytes about 5-10% of the total LDH leaks into the extracellular medium during a 4-hr incubation period. Human hepatocytes lose at least 20% of the total LDH to the medium, and some preparations release as much as 60-80% in 4 hr. This observation led us to conduct a study that compared the metabolism of a test compound, mofezolac, by suspension and monolayer cultures of human hepatocytes (Green et al., 1993). In that study, human hepatocyte monolayer

cultures metabolized mofezolac with a higher V_{\max} (similar to that obtained with human liver microsomes) and formed a more complete metabolite profile than human hepatocyte suspension cultures. In contrast, no difference was found in the rate of benzo(a)pyrene metabolism by rat hepatocytes in suspension and monolayer cultures (Knadle et al., 1992). Unfortunately, monolayer cultures are probably not a practical alternative system for the present studies. Volatile compounds are very difficult to handle in monolayer cultures because the halogenated solvents dissolve into the plastic of the culture dishes and hepatocytes do not attach to glass culture vessels. Therefore, we decided to investigate precision-cut liver slices, an alternative *in vitro* system developed as a model for metabolism studies and reported to be useful with human and rat liver specimens.

HEPATOCYTES

In our earlier studies, we had added the volatile test chemicals to a center-well in the flask and allowed them to equilibrate between the air and media phases (Tyson et al., 1983; Knadle et al., 1990). However, in the current experiments it was necessary to work with lower concentrations of the chlorinated solvents. Therefore, we prepared stock solutions of chloroform in sealed vials and then added an aliquot to the incubation vials. The experiments started in the first year of the project on chloroform metabolism by rat hepatocytes were continued in the second year to more completely characterize this *in vitro* model.

The time course of metabolism of chloroform at 2 to 4 different concentrations by hepatocytes was determined in several experiments. The headspace of the incubation flasks was sampled at repeated time points to determine the loss of the parent compound. The complete data set for these experiments is included in the Appendix.

One problem encountered in these experiments has been in the quantitative determination of chloroform content. In several experiments, attempts to determine the level of chloroform in the medium failed because of an interference in the incubation medium that resulted in unreasonably high values. In some more recent experiments, the values obtained for the headspace concentration of chloroform have been higher than anticipated (e.g., see Appendix Table A-5, 72 nmol/flask, and Table A-6, 7.2 nmol/flask). These higher values may be simply due to the difficulties inherent in quantitatively transferring and sampling a volatile solvent in a 37°C incubation system. However, since we have had extensive experience handling such chemicals in the past, we are carefully examining the gas chromatography equipment and reviewing the procedures for preparing standard curves. The level of chloroform in the samples analyzed in these experiments has varied widely, making it difficult to keep all samples within the linear range of the standard curve. Cross contamination between samples can also be a problem in these experiments when three different concentrations ranging over three orders of magnitude are studied at the same time with very close time-points for

analysis. Each of these questions is being carefully considered in all future experiments to maximize the accuracy of the results.

In general, in the time-course experiments, we found that within about 5 min the chloroform added to the medium had equilibrated with the headspace. The concentration of chloroform in the headspace of flasks containing heat-killed cells was stable during the incubation period (Figure 1). Figure 2 shows a graph of the time course of chloroform loss from the headspace of rat hepatocytes incubated with 7.2 nmol/flask (actual measured concentration was 6.5 ± 2.5 nmol/flask). The results from four different experiments are plotted on the same graph to show that the data were reproducible in repeat experiments. The changes in chloroform concentration in both the headspace and the media were compared in another experiment, and the results are shown in Figure 3. In this experiment, we observed that the concentration of chloroform changed in both the headspace and the medium so that either compartment could be analyzed to determine the rate of metabolism. However, because a larger fraction of the total dose of chloroform was found in the air than in the medium, sampling the headspace should provide greater sensitivity in the analysis, as well as offer a convenient and rapid analysis method as documented by other laboratories as well (Sato and Nakajima, 1979).

Figure 4 is a Lineweaver-Burk plot of the loss of chloroform from the headspace of hepatocyte incubations. From the results of this analysis, we estimated the kinetic constants for the metabolism of chloroform by rat hepatocytes to be $K_m = 34$ nmol/flask and $V_{max} = 24$ pmol/min/ 10^6 cells. Assuming that the chloroform is evenly distributed in the flask and that the total volume is 26 ml, the K_m is approximately $1.3 \mu M$.

LIVER SLICES

We also examined rat liver slices for their ability to metabolize chloroform. Figure 5 shows the results of two experiments in which 3.6 and 36 nmol/flask were studied. At the lower concentration, approximately half of the initial concentration of chloroform was lost in 30 min or less, whereas at the higher concentration a significant decrease in the chloroform was not detected until 60 min.

COMPARISON OF ECOD ACTIVITY IN HEPATOCYTES AND SLICES

Very little work has been done to directly compare isolated hepatocytes and liver slices to determine which of these *in vitro* models best represents the intact organ. In fact, two published studies provide conflicting information. Berthou et al. (1989) investigated caffeine metabolism by human liver microsomes, hepatocyte cultures, and liver slices and found that the rate of metabolism by the liver slices was much lower than that of hepatocytes. In contrast, Powis et al. (1989) found that biphenyl metabolism,

although similar in rat hepatocytes and liver slices, was significantly lower in human and dog hepatocytes compared to slices prepared from the same tissues. Unfortunately, the interpretation of these data is difficult because neither laboratory used precision-cut liver slices in organ culture, a technique that has been documented to be superior to the less reproducible, thicker slices cut by hand (Smith et al., 1986). In addition, Powis and colleagues isolated the hepatocytes by a nonperfusion method and incubated them in suspension culture, both of which have been shown to lead to liver cells of reduced functionality and longevity (Gustavsson and Morland, 1980; Sturdee et al., 1983; Green et al., 1993).

Therefore, we studied the metabolism of a model substrate, ethoxycoumarin (EC), in hepatocytes in monolayer culture and precision-cut liver slices from both rat and human liver. Figure 6 illustrates the results of an experiment with rat liver preparations in which the stability of metabolism over 4 hr was determined. ECOD activity did not change significantly in either rat hepatocyte or liver slice incubations under these conditions. Table 1 summarizes the results of our comparison of liver slices and hepatocytes from both rat and human liver. With rat liver tissue we found that the hepatocyte-to-slice ratio of ECOD activity (standardized on the basis of protein content) was about 3. This result is not totally unexpected because hepatocytes constitute only about 60% of the total cell number in the liver. With human liver tissue the ratio of hepatocyte-to-slice ECOD activity varied from 4.5 to 12.6. The significantly higher activity in isolated human hepatocytes suggests that human liver slices contain numerous metabolically inactive cells, either a larger fraction of nonparenchymal cells or more nonviable parenchymal cells as compared to rat liver. However, human liver slices converted a larger fraction of the primary metabolite, HC, to conjugates than did human hepatocytes, suggesting that liver slices better maintain synthesis capabilities than do liver cells in culture. Thus, both preparations have certain advantages over the other.

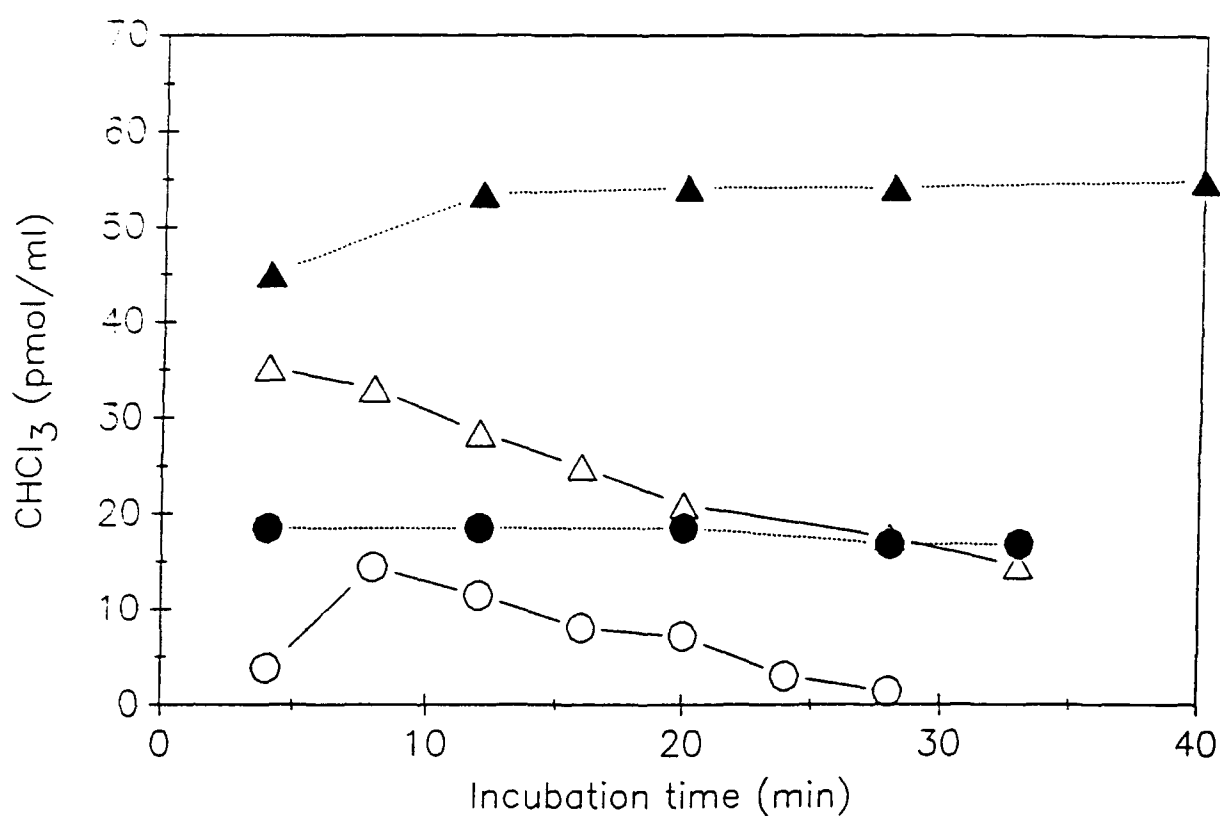


Figure 1. Headspace analysis of chloroform in rat hepatocytes. Chloroform was incubated with 4×10^6 cells/ml in an air-tight flask with a total volume of 26 ml (4 ml of medium and 22 ml headspace). Circles = 0.72 nmol/flask; triangles = 7.2 nmol/flask; solid line plots are for live cells and dashed line plots are for heat-killed cells.

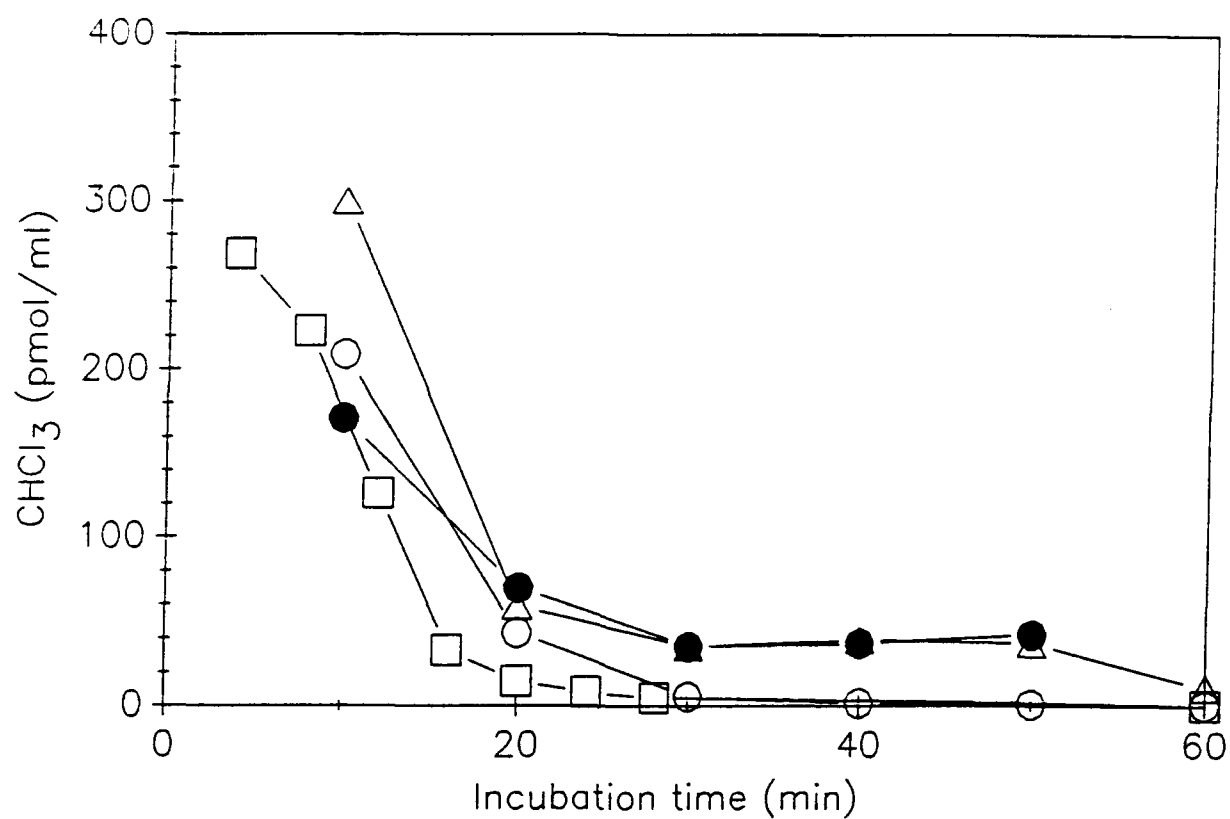


Figure 2. Headspace analysis of chloroform in rat hepatocytes from four different experiments. Chloroform was incubated with 4×10^6 cells/ml in an air-tight flask with a total volume of 26 ml (4 ml of medium and 22 ml headspace). Hepatocytes were incubated with 7.2 nmol/flask (actual measured concentration was 6.5 ± 2.5 nmol/flask).

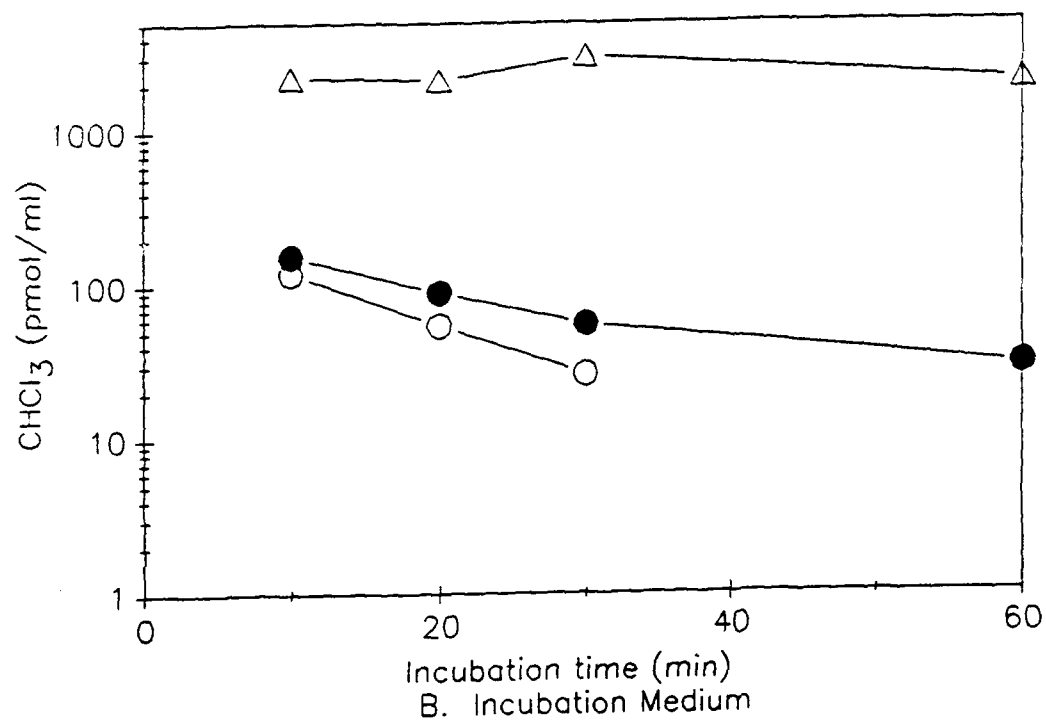
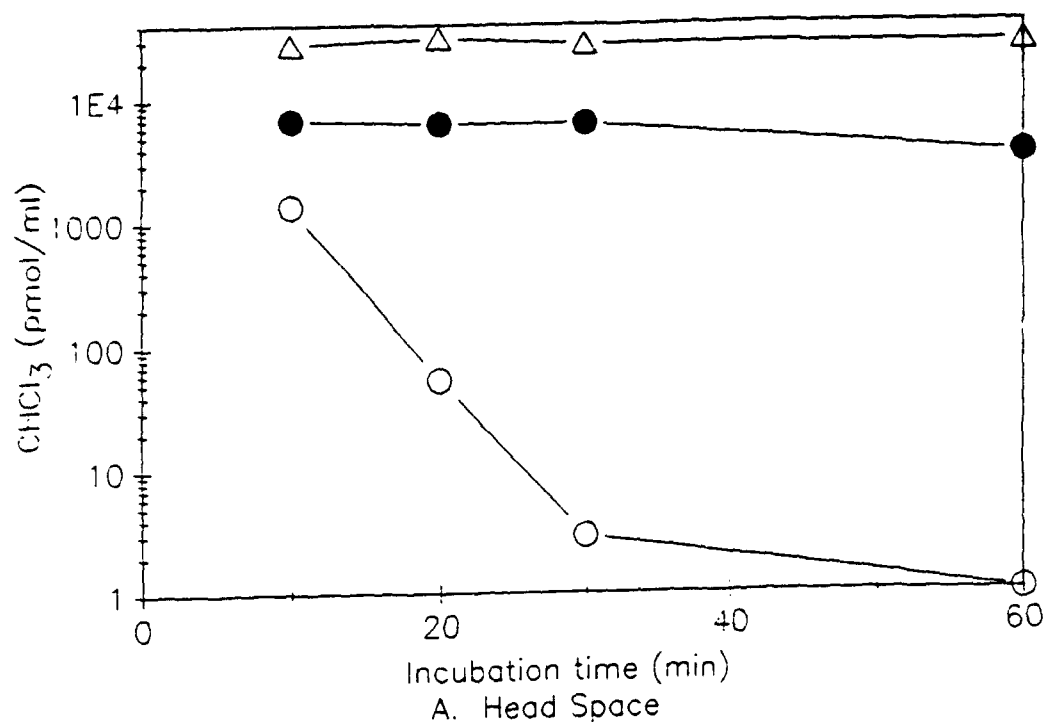


Figure 3. Change in chloroform concentration in incubations with rat hepatocytes. Chloroform was incubated with 4×10^6 cells/ml in an air-tight flask with a total volume of 26 ml (4 ml of medium and 22 ml headspace). Hepatocytes were incubated with 7.2 (open circles), 72 (closed circles), and 720 (open triangles) nmol/flask. A. Headspace analysis of chloroform; B. Incubation medium analysis of chloroform.

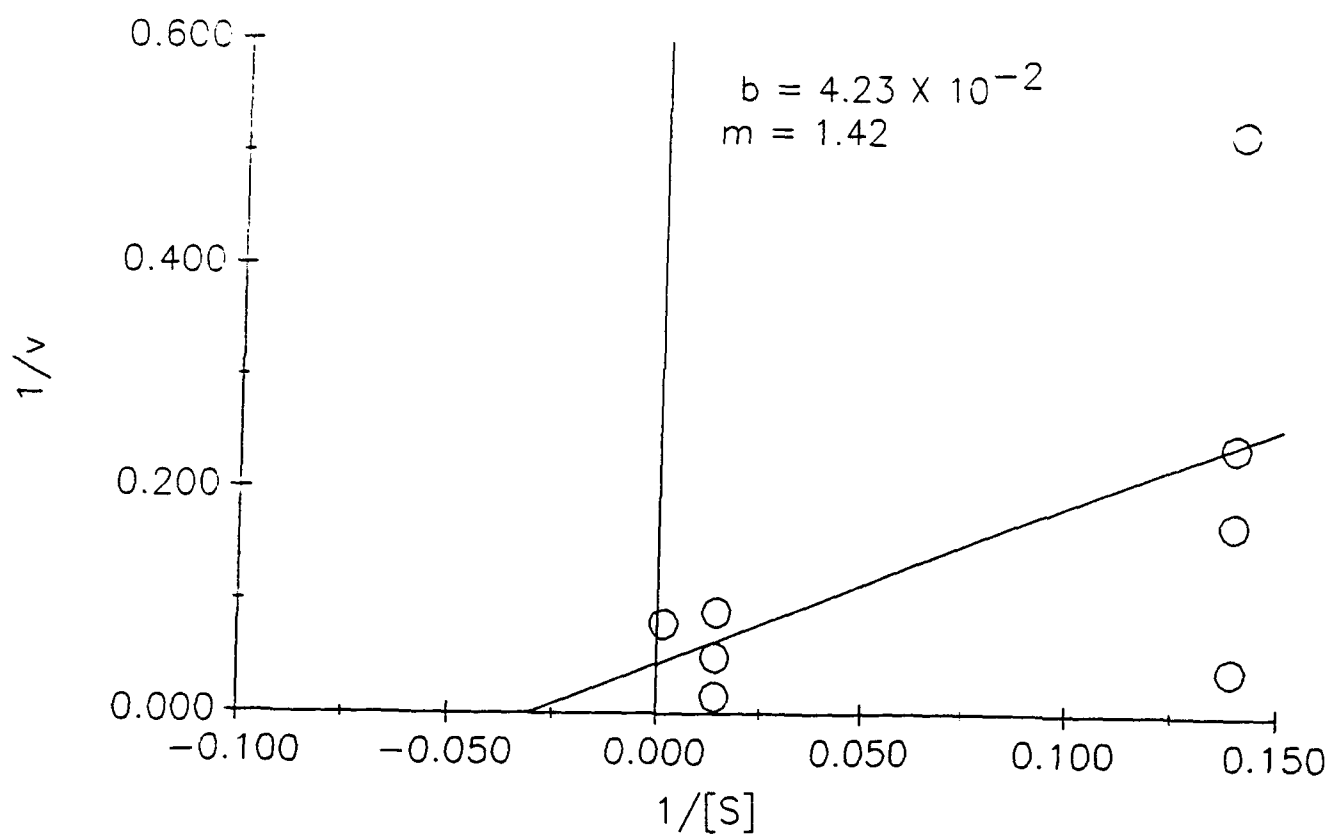


Figure 4. Lineweaver-Burk double-reciprocal plot of substrate concentration (chloroform) versus the rate of loss of chloroform from the headspace. The y-intercept of the regression line is "b" and the slope is "m".

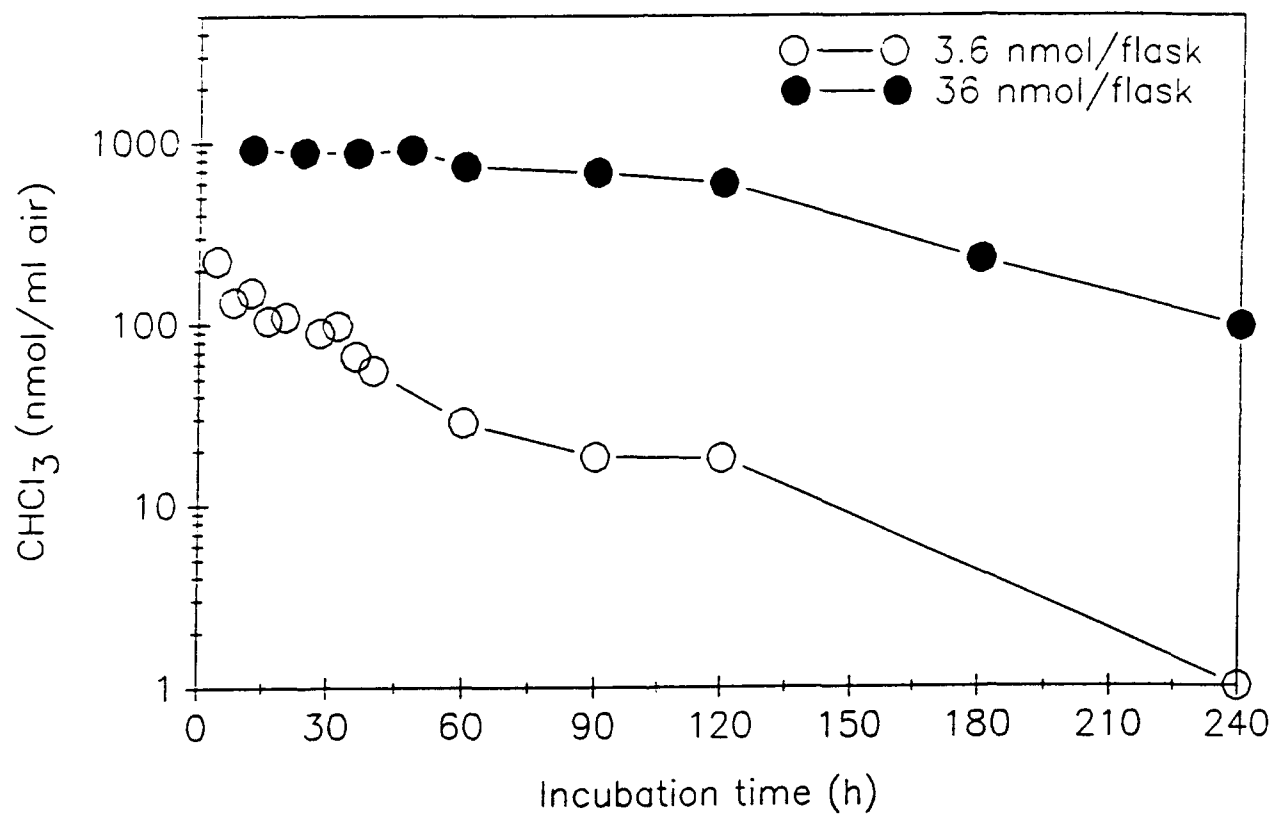


Figure 5. Headspace analysis of chloroform in rat liver slices. Chloroform was incubated with 4 liver slices in a gas-tight flask with a total volume of 24 ml (2 ml of medium and 22 ml headspace). Open circles = 3.6 nmol/flask; closed circles = 36 nmol/flask.

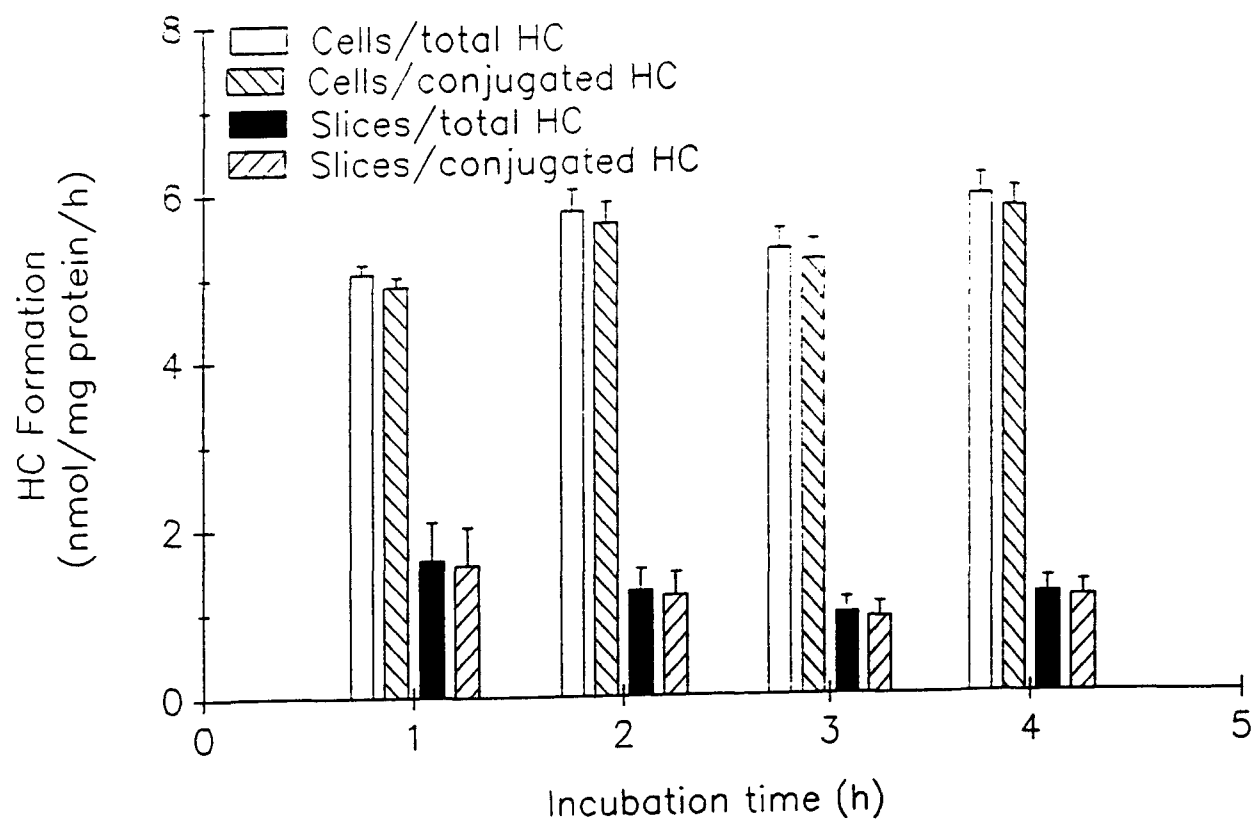


Figure 6.

Comparison of ECOD activity in rat hepatocytes and rat liver slices. Both preparations were incubated with 100 μ M EC for 1 hr and the amount of HC, total and conjugated, was determined.

Table 1
COMPARISON OF ECOD ACTIVITY IN HEPATOCYTES AND LIVER SLICES

<u>In Vitro Preparation</u>	<u>ECOD (nmol/mg protein/hr)</u>	<u>Conjugation (% of total)</u>	<u>ECOD Cells/slices</u>
Rat			3.1
Hepatocytes	5.04 ± 0.12	97.2 ± 0.06	
Liver slices	1.63 ± 0.46	96.2 ± 0.60	
Human (H-81)			12.6
Hepatocytes	5.06	69.2	
Liver slices	0.403	87.0	
Human (H-84)			4.5
Hepatocytes	3.81	19.7	
Liver slices	0.856	86.8	
Human (H-86) ^a			9.3
Hepatocytes	22.8	66.0	
Liver slices	2.44	92.8	

^aSpecimen H-86 was recovered from a patient who had taken phenobarbital for about 6 months before his death.

PLANS FOR THE THIRD YEAR

Some of the studies originally planned for the second year of the project will now be performed in the third year, in addition to tasks scheduled for the third. The following experiments are planned:

- The rate of chloroform loss from the headspace of rat liver slices will be determined at several different concentrations of chloroform, and the kinetic constants will be calculated.
- Because human hepatocytes lose viability rapidly in suspension and because monolayer cultures are not a practical alternative for studies with volatile hydrocarbon solvents, human liver slices will be studied. The metabolism of chloroform by human and rat precision-cut liver slices will be compared.
- Metabolism of at least three of the other halogenated solvents originally proposed (carbon tetrachloride, trichloroethylene, dichloromethane, 1,1,1-trichloroethane), and bromochloromethane will be studied in the *in vitro* model, and the kinetic constants will be determined. The values obtained will be compared to published values determined *in vivo*. The incubation conditions will be modified as needed to obtain a good correlation between the *in vivo* and *in vitro* results.

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Appendix

**DATA FROM EXPERIMENTS ON THE METABOLISM OF CHLOROFORM
BY RAT HEPATOCYTES AND LIVER SLICES**

Table A-1
METABOLISM OF CHLOROFORM BY RAT HEPATOCYTES (EXP RH5)
HEADSPACE ANALYSIS

<u>CHCl₃ Added (nmol/flask)</u>	<u>Incubation Time (min)</u>	<u>Live Cells (CHCl₃ pmol/ml)</u>	<u>Heat-Killed Cells (CHCl₃ pmol/ml)</u>
0.72	4	3.8	18.5
	8	14.4	----
	12	11.4	18.5
	16	8.0	----
	20	7.1	18.5
	24	3.0	----
	28	1.4	16.8
	40	---	16.8
7.2	4	35.2	45.0
	8	33.0	---
	12	28.4	53.4
	16	24.9	---
	20	20.9	54.1
	28	17.5	54.1
	33	14.5	---
	40	---	54.8
7.2	4	39.7	
	8	35.5	
	12	60.0	
	16	58.3	
	20	54.0	
	24	51.5	
	28	49.7	
	35	45.9	
	40	42.8	

Table A-2
METABOLISM OF CHLOROFORM BY RAT HEPATOCYTES (EXP RH6)
HEADSPACE ANALYSIS

<u>CHCl₃ Added (nmol/flask)</u>	<u>Incubation Time (min)</u>	<u>CHCl₃ in Air (pmol/ml)</u>
0.72	10	1.08
	20	0.194
	30	0.055
	40	0.031
	50	0.040
	60	0.026
7.2 ^a	10	42.0
	20	31.0
	30	19.3
	40	13.8
	50	9.72
	60	5.52

^aValues represent the mean of determinations from duplicate incubation flasks.

Table A-3
METABOLISM OF CHLOROFORM BY RAT HEPATOCYTES (EXP RH7)
HEADSPACE ANALYSIS

<u>CHCl₃ Added^a</u> <u>(nmol/flask)</u>	<u>Incubation</u> <u>Time (min)</u>	<u>CHCl₃ in air</u> <u>(pmol/ml)</u>
0.072	10	0.977
	20	0.786
	30	0.577
	40	0.741
	50	0.482
	60	0.232
0.72	10	4.64
	20	1.24
	30	0.877
	40	0.786
	50	0.632
	60	---
7.2	10	209
	20	43.2
	30	5.36
	40	1.48
	50	0.68
	60	0.63

^aStock solutions were analyzed for chloroform and were found to contain chloroform concentrations such that 0.059, 0.51, and 3.99 nmol/flask were added to the incubations.

Table A-4
METABOLISM OF CHLOROFORM BY RAT HEPATOCYTES (EXP RH8)
HEADSPACE ANALYSIS

<u>CHCl₃ Added^a</u> <u>(nmol/flask)</u>	<u>Incubation</u> <u>Time (min)</u>	<u>CHCl₃ in Air^b</u> <u>(pmol/ml)</u>
7.2	10	170
	20	113
	30	35.1
	40	36.8
	50	42.1
	60	--- ^c
72	10	2464
	20	1655
	30	1713
	40	1195
	50	1102
	60	866
720	10	9524
	20	8991
	30	6049
	40	3434
	50	--- ^c
	60	--- ^c

^aStock solutions were analyzed for chloroform and were found to contain chloroform concentrations such that 9.9, 62, and 805 nmol/flask were added to the incubations.

^bValues are the mean of determinations from duplicate incubations flasks.

^cSampling error at these time points.

Table A-5
METABOLISM OF CHLOROFORM BY RAT HEPATOCYTES (EXP RH9)
HEADSPACE ANALYSIS

<u>CHCl₃ Added^a</u> <u>(nmol/flask)</u>	<u>Incubation</u> <u>Time (min)</u>	<u>CHCl₃ in Air^b</u> <u>(pmol/ml)</u>
7.2 ^c	10	300
	20	59.0
	30	35.4
	40	39.3
	50	3.7
	60	1.1
72	10	10716
	20	8084
	30	7460
	40	4850
	50	5386
	60	4116
720	10	32935
	20	41302
	30	32091
	40	38406
	50	43335
	60	30848

^aStock solutions were analyzed for chloroform and were found to contain chloroform concentrations such that 6.5, 63, and 624 nmol/flask were added to the incubations.

^bValues are the mean of determinations from duplicate incubations flasks.

^cValues for 7.2 nmol/flask are single determinations because the duplicate was not gas-tight.

Table A-6
METABOLISM OF CHLOROFORM BY RAT HEPATOCYTES (EXP RH10)
HEADSPACE ANALYSIS

<u>CHCl₃ Added^a</u> <u>(nmol/flask)</u>	<u>Incubation</u> <u>Time (min)</u>	<u>Live Cells</u> <u>(pmol/ml)</u>	<u>Heat-Killed Cells</u> <u>(pmol/ml)</u>
7.2	10	1340	1038
	20	53.6	1013
	30	2.9	946
	60	0	982
72	10	6740	7346
	20	6285	6880
	30	6273	7693
	60	3414	7401
720	10	27669	26727
	20	31119	27443
	30	27799	33126
	60	27615	33778

^aStock solutions were analyzed for chloroform and were found to contain chloroform concentrations such that 5.5, 52, and 625 nmol/flask were added to the incubations.

Table A-7
METABOLISM OF CHLOROFORM BY RAT HEPATOCYTES (EXP RH10)
MEDIA ANALYSIS

<u>CHCl₃ Added^a</u> <u>(nmol/flask)</u>	<u>Incubation</u> <u>Time (min)</u>	<u>Live Cells</u> <u>(pmol/ml)</u>	<u>Heat-Killed Cells</u> <u>(pmol/ml)</u>
7.2	10	120	40.9
	20	54.4	47.0
	30	26.2	39.4
	60	--- ^a	86.6
72	10	154	172
	20	89.6	160
	30	55.4	178
	60	29.5	177
720	10	2223	2117
	20	2133	2545
	30	3027	2520
	60	2074	2160

^aStock solutions were analyzed for chloroform and were found to contain chloroform concentrations such that 5.5, 52, and 625 nmol/flask were added to the incubations.

Table A-8
METABOLISM OF CHLOROFORM BY RAT LIVER SLICES (RS1)^a
HEADSPACE ANALYSIS

<u>CHCl₃ Added (nmol/flask)</u>	<u>Incubation Time (min)</u>	<u>Live Slices (pmol/ml)</u>	<u>Heat-Killed Slices (pmol/ml)</u>
3.6	4	224	---
	8	133	---
	12	150	---
	16	105	---
	20	110	---
	28	89.8	---
	32	97.8	---
	36	66.3	---
	40	55.2	---
	60	28.6	---
	90	18.5	147
	120	18.2	---
	240	0	---
36	10	768	---
	20	614	---
	40	813	---
	60	676	---
	90	---	804

^aEach flask contained 4 liver slices that weighed 200 to 238 mg/4 slices.

Table A-9
METABOLISM OF CHLOROFORM BY RAT LIVER SLICES (RS2)^a
HEADSPACE ANALYSIS

<u>CHCl₃ Added (nmol/flask)</u>	<u>Incubation Time (min)</u>	<u>Live Slices^b (pmol/ml)</u>	<u>Heat-Killed Slices (pmol/ml)</u>
36	12	921 ± 22.9	---
	15	---	934 ± 55.2 ^b
	24	884 ± 61.1	---
	30	---	971 ^c
	36	875 ± 50.0	---
	48	913 ± 43.9	---
	60	737 ± 15.7	---
	90	680 ± 36.2	---
	120	595 ± 39.8	980 ^c
	180	225	---
	240	97 ± 17.3	---

^aEach flask contained 4 liver slices that weighed 125 to 170 mg/4 slices.

^bValues represent the mean ± sd of determinations from triplicate incubation flasks.

^cValues represent the mean of determinations from duplicate incubation flasks.